



Description of the tadpole and natural history notes of *Incilius spiculatus* (Mendelson, 1997), an Endangered toad endemic to the Sierra Madre de Oaxaca, Mexico

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Abstract.—Amphibian populations are declining rapidly around the world. However, new amphibian species keep being discovered, reflecting the still expanding state of our knowledge of this group. Similarly, there is a lack of information regarding life cycles, particularly among those species that have indirect development with a free-living larval stage. Many amphibian larvae are still unknown or undescribed, thus impeding a proper understanding of the biology and habitat use of many species. In this paper, we describe the tadpole of the Endangered bufonid anuran *Incilius spiculatus*, a member of a clade known as forest toads. Also described are the amplexus behavior of this species observed in nature, and aspects of the natural history of the adult stage. A tadpole identification key is provided for the forest toad clade of Mexico and Central America. This information contributes to the understanding of the life history of *I. spiculatus*, in addition to its diet and distributional patterns. This article highlights the importance of knowing the complete life cycle of a species in order to establish effective conservation plans, particularly for those species with limited distributions in highly managed ecosystems.

Keywords. Anura, forest toads, *valliceps* group, amplexus, stream breeding, scorpion predation

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Introduction

In recent years, the continuing discovery of new amphibian species (Canseco-Márquez et al. 2017; Jiménez-Arcos et al. 2019; Parra-Olea et al. 2020) and larval phases (Downie et al. 2015; Kaplan and Heimes 2015; Köhler et al. 2015) has revealed the limited state of our knowledge regarding amphibians, which are currently the vertebrate group with the highest risk of extinction (Beebe and Griffith 2005; IUCN 2020; Scheele et al. 2019; Stuart et al. 2008).

A similar situation is the lack of information that exists regarding life cycles, in particular for those species that exhibit indirect development with a free-living larval stage. These species usually inhabit different habitats throughout their life cycle and play different ecological roles in relation to their development stages. For example, most tadpoles contribute to maintaining healthy water bodies by consuming algae and breaking down organic material (Cortés-Gomez et al. 2015), while transferring

energy between aquatic and terrestrial habitats (Flecker et al. 1999; Kupferberg 1997; Regestert et al. 2008). As adults, these organisms usually prey on invertebrates, thus controlling their populations, while at the same time acting as prey for other organisms (Duellman and Trueb 1994; Stewart and Woolbright 1996). This duality also means that each life-history stage is exposed to different risk factors. Thus, when aiming to conserve a species with a biphasic life cycle, it is essential that we have thorough knowledge of both life-history stages.

Toads of the genus *Incilius* are anurans with a biphasic life cycle which inhabit the Americas. Their geographical distribution ranges from the southern edge of the United States to northern Colombia (Mendelson et al. 2011). Currently the genus contains 40 recognized species, of which 23 have a distribution within Mexico (AmphibiaWeb 2020; Wilson et al. 2013; Schachat et al. 2016). Among these are the so-called forest toads, a clade that groups various species that inhabit wet forests and are sensitive to habitat transformation as detected in their

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adult stage (Mendelson et al 2011). Despite this known sensitivity, information on the larval stages of these toads is incomplete. The forest toad group is composed of 10 species: *Incilius aucoinae*, *I. melanochlorus*, *I. cristatus*, *I. macrocristatus*, *I. cavifrons*, *I. spiculatus*, *I. tutelarius*, *I. aurarius*, *I. leucomyos*, and *I. campbelli*, among which the larvae of only seven species have been described (Mendelson et al. 2012; Segura-Solis and Bolaños 2008). The larvae of *I. melanochlorus*, *I. campbelli*, and *I. spiculatus* are unknown, reflecting the fact that even though the description of anuran tadpoles from Latin America is increasing, there are still many Neotropical species whose larval stage remains to be described (Downie et al. 2015; Kaplan and Heimes 2015; Köhler et al. 2015).

During recent surveys in the northern slopes of the Sierra de Juárez in Oaxaca, we found an amplexant pair of *Incilius spiculatus*, an endemic species of southeast Mexico that is restricted to the highlands of the physiographic sub-province of the Sierra Madre de Oaxaca (Mendelson 1997; Ortiz-Pérez et al. 2004). This species occurs mainly in montane cloud forests and is listed as Endangered (EN) according to the IUCN (IUCN 2020). It was originally described from adult specimens deposited in scientific collections; however, its breeding behavior has not been recorded nor has the tadpole been described, despite extensive fieldwork conducted in the region (Caldwell 1974; Caviedes-Solis et al. 2015; Delia et al. 2013; Lips et al. 2004; Mendelson 1997). In addition, many aspects of the natural history and reproductive biology of *I. spiculatus* are still unknown, probably due to taxonomic confusion of this species with *I. cristatus* and *I. valliceps*, as well as its restricted distribution and lack of field observations (Mendelson 1997).

In this paper, the tadpole of *I. spiculatus* is described and information on breeding sites, amplexus type, and clutch size are documented. An identification key for the tadpoles of the forest toad clade (Mendelson et al. 2011) in Mexico and Central America is provided, in addition to observations on adult *I. spiculatus* with comments on conservation implications that might allow this endangered toad to persist in the region.

Materials and Methods

Surveys were conducted at San Pedro Yolox (17.589359°N, -96.551790°W; datum WGS84) and Santa Cruz Tepetotutla (17.739446°N, -96.558292°W), both located on the northern slopes of the Sierra Juárez, Oaxaca, Mexico, within the sub-physiographic province Sierra Madre de Oaxaca (Ortiz-Pérez et al. 2004).

In February 2019, a pair of *I. spiculatus* was found in amplexus and laying eggs at Rio Coyul, San Pedro Yolox (17.64015°N, -96.4306°W, 645 m asl). The species was identified according to the reported distribution and morphological characteristics described by Mendelson (1997), particularly the presence of cranial crests, spiculate tubercles on the limbs, and pale fingertips. The female body temperature and clutch temperature

were taken with an infrared thermometer. Seventy eggs were collected from the egg clutch without disturbing the adult toads (permit FAUT-0074) and taken to the Amphibian Ecology Laboratory at Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca (CIIDIR-Oaxaca). The eggs were kept in glass tanks with aerated water. The water was partially replaced every two weeks and tadpoles were fed boiled spinach and lettuce *ad libitum*. The room temperature ranged from 23 to 30 °C. Tadpoles were euthanized with 5% lidocaine and preserved in 10% formalin.

A total of 34 tadpoles at stages 26 to 37 (Gosner 1960) were examined using a microscope (Carl Zeiss 2000-C) and photographed (Canon Powershot GX5) for their subsequent measurements. For tadpole morphology, the terminology of Altig (1970, 2007) was followed. Measurements were made with tpsUtil and tpsdig2 software (Rohlf 2017, 2019). Photographs of the oral apparatus were taken specifically at Gosner stages 24 and 37. The oral formula followed Altig and McDiarmid (1999). Live coloration and codes were described following Köhler (2012). All specimen series were deposited in the Museo de Zoología Facultad de Ciencias (MZFC35365, MZFC35366, MZFC35367) at the Universidad Nacional Autónoma de México. Larval development time under laboratory conditions was measured by recording the progress between the 46 Gosner stages until full metamorphosis was reached.

Clutch size. The approximate total number of eggs in the clutch was calculated by multiplying the average number of eggs in 10 cm sections of the egg string (counted at five different sections) by the total length of the egg string. The total length was an approximate measurement to avoid disturbing the clutch. In addition, air and water temperatures were taken, and the river width was measured with a flexometer.

Adult diet. In order to identify items in the diet, six adult toads were collected in the field and kept in plastic boxes with access to water until they defecated, after which they were released. The fecal samples were preserved in 70% alcohol and examined with a microscope (Leica model EZ4 stereoscopic). Food items or structures were identified with specialized insect keys (Palacios-Vargas et al. 2014; Ríos-Casanova 2014; Vélez and Vivallo 2018).

Results

Tadpole Description

Average measurements (mm) for Gosner stage 35: body length 13.28, tail length 21.68, tail muscle height 3.41, maximum tail height 6.09, total length 34.96, tail muscle width 3.31, internarial distance 1.66, interorbital distance 4.70; measurements for other Gosner stages are presented in Table 1. The body is

Table 1. Measurements of the tadpole of *Incilius spiculatus* by Gosner stage (averages in mm). Body length (BL), Tail length (TAL), Tail muscle height (TMH), Maximum tail height (MTH), Total length (TL), Tail muscle width (TMW), Internarial distance (ID), Interorbital distance (IOD).

Stage	Lateral view				Dorsal view			
	BL	TAL	TMH	MTH	TL	TMW	ID	IOD
26	6.25	9.87	1.38	2.85	16.12	1.00	0.92	1.87
27	6.08	9.15	1.53	2.84	15.23	1.10	0.92	1.79
28	6.61	9.84	1.52	3.22	16.45	1.27	1.15	2.25
29	8.04	11.20	1.82	3.91	19.25	1.38	1.15	2.26
30	8.95	12.12	1.95	4.26	21.07	1.55	1.26	2.57
31	8.63	11.77	1.75	4.16	20.40	1.45	1.28	2.50
32	8.51	12.80	2.12	4.38	21.31	1.52	1.32	2.49
33	9.03	13.61	2.74	4.63	22.63	1.68	1.28	2.69
34	8.79	13.60	2.14	4.59	22.39	1.66	1.32	2.62
35	13.28	21.68	3.41	6.09	34.96	3.31	1.66	4.70
36	12.89	21.76	3.29	5.90	34.66	2.81	1.59	4.12
37	13.90	22.73	3.44	6.29	36.63	3.05	1.70	4.84

ovoid in dorsal view, widest at about the middle point and narrower near the tail; depressed in lateral view. Snout nearly semicircular in dorsal profile, rounded at the tip in lateral profile. Spiracle sinistral with inner wall free from body. External nares ovoid situated nearer to eyes than to snout. Eyes dorsal. Vent tube medial. Tail rounded at the tip. Caudal musculature highest at base, gradually tapering to a pointed tip; dorsal fin reticulated (Fig. 1).

Oral disk small; labial tooth row formula 2(2)/3, A1 slightly longer than other rows; A2 gap narrow, approximately width of 3–8 teeth, P1 and P2 equal in length, P3 being the longest posterior row; labial papillae restricted to lateral portions of oral disc disposed in two interposed series (Fig. 1A). At stage 24, the larvae show an elongation of the oral apparatus that protrudes from the body (Fig. 2D–E).

In life, the color of the body is uniformly Cinnamon Brown (color 43 in Köhler 2012), except for the ventral part of the mouth where the color becomes paler. The venter is slightly transparent, with a counter-clockwise coiled intestine visible. Around Gosner stage 35, small Cream Yellow (82) dots appear throughout the body and the iris (Fig. 2G). The tail fins are transparent with large Cinnamon Brown (43) pigment granules forming a reticulate pattern on the dorsal fin. Around Gosner stage 37, the ventral parts of the limbs are pale Cinnamon Brown (43) with Cream Yellow (82) dots and dark brown bars dorsally. In preservative, the tadpole body and the tail musculature are Natal Brown (49), while the ventral part of the body is slightly translucent.

Tadpole development required approximately 35 days to complete metamorphosis under laboratory conditions (Fig. 2H). Three days after collection (8 February 2019), embryonic development reached Gosner stage 12. Three days later, the embryos had reached stage 18, and four days after that had developed into stage 25 tadpoles.

Adult Breeding Behavior

In February 2019, an amplexing pair of *Incilius spiculatus* was observed at 640 m asl in the shallower margins of a river (Río Coyul), where the water current was slowed by the presence of rocks and aquatic vegetation. The Río Coyul is a permanent river with an average width of 8.19 m at the site where the amplexus was observed (Fig. 2F).

The toads used vegetation and material on the bottom of the river to maintain their position in the water during the amplexus. Mating was observed during the day and the amplexus was axillary (Fig. 2A–B). At the time of the observations (1250 h GST), the water temperature was 19.6 °C. The species exhibited an ovipositional string mode with a double row of eggs arrangement (Altig and McDiarmid 2007). The estimated clutch size was 4,500 eggs, and the clutch was attached to aquatic vegetation on rocks at the river margins at about 35 cm depth (Fig. 2C). Amplexant behavior was observed from the time of encounter until the toads separated (approximately 2.67 h). The female body temperature was 21.0 °C, SVL 85.5 mm, and the clutch temperature was 21.0 °C. In addition to the amplexant pair, a second male toad was observed in the water at a distance of 1.0 m. A second egg clutch was observed in another pool located 3.70 m away; but the toads that laid this clutch were not observed.

Adult Diet

Fecal samples indicated that Hymenoptera were the dominant prey (15 individuals, 48.3%), followed by Coleoptera (7, 22.5%), Scorpiones (6, 19.3%), Orthoptera (2, 6.4%), and Blattodea (1, 3.2%).

Extension of Elevational Range

Previously, the elevational range reported for *I. spiculatus* was from 800–1,689 m asl (Mendelson 1997). During this survey, two juvenile individuals were observed at

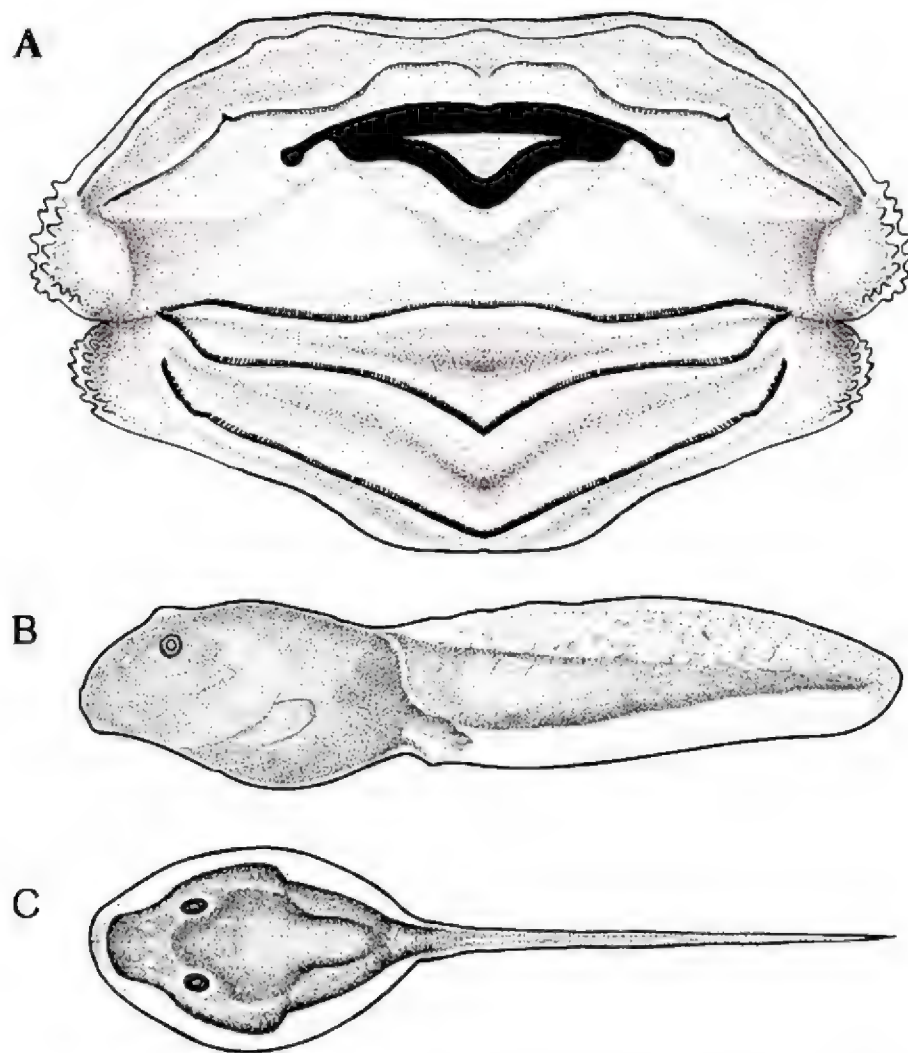


Fig. 1. Tadpole of *Incilius spiculatus*. (A) Oral disc at Gosner stage 37, (B) lateral view at Gosner stage 35, and (C) dorsal view at Gosner stage 35.

Santa Cruz Tepetotutla, Oaxaca, Mexico, in a patch of primary cloud forest vegetation at an elevation of 1,758 m asl (17.71862°N, -96.55911°W) datum WGS84, in addition to three individuals in San Pedro Yolox at 682 m asl (17.63622°N, -96.42735°W), 643 m asl (17.64001°N, -96.43061°W), and 642 m asl (17.64013°N, -96.43056°W). With these records, the altitudinal range of this species is now extended as including from 642 to 1,758 m asl.

Discussion

The observations reported here confirm that *Incilius spiculatus* is a stream breeder, and that its reproduction occurs during the dry season, as in the other species in the forest toad group (Mendelson et al. 2011). With this description, the number of known forest toad tadpoles is increased to eight; the only species yet to be described are *I. melanochlorus* and *I. campbelli* (Altig 1970, 1987; Korky and Webb 1973; Mendelson et al. 1999, 2012; Segura-Solis and Bolaños 2009; Shannon and Werler 1955). Among the tadpoles of this group, *I. tutelarius*, *I. macrocristatus*, *I. leucomyos*, and *I. spiculatus* share the oral formula 2 (2) / 3, as does *I. valliceps* (Korky and Webb 1973; Limbaugh and Volpe 1957; McCranie and David 2000; Mendelson et al. 1999). Although *I. valliceps* is not a forest toad, it is included in the identification key here because it is sympatric with *I. spiculatus* and the two could be easily confused due to similar morphology. The difference between the two species is that *I. spiculatus* has an A2 gap that is 3–8 teeth wide, and it has a reticulated pattern only on its dorsal fin.

A peculiar morphological characteristic of *I. spiculatus* present at Gosner stage 24 is an oral apparatus that protrudes from the body (Fig. 2D–E). As far as we know, this feature has not been reported for any other species of anurans. A limiting factor for comparison with other species is that most descriptions are made from tadpoles at developmental stages beyond Gosner stage 25, so larval development in earlier stages is generally unknown. The closest related species for which a complete larval development description (from fertilization to metamorphosis) could be found is *I. valliceps*, and it does not have this oral morphology type (Limbaugh and Volpe 1957). Descriptions of earlier tadpole development stages should be encouraged because they have proven to be useful for differentiating species in groups where the morphological variations of larvae in advanced stages (beyond Gosner stage 34) are almost non-existent (Laufer et al. 2013). In addition, we consider that these observations can contribute to the understanding of larval ecology. As mouth shape is known to be associated both with habitat type and diet in other amphibians (Altig and McDiarmid 1999; Van-Buskirk 2009), we suspect that this structure might be either an adaptation to life in flowing water that prevents tadpoles from being washed away (by allowing them to attach to fixed material) or a foraging adaptation in early development that changes in the later stages. The diet type at this stage should be investigated to further elucidate its function.

In relation to breeding behavior, the findings reported here confirm that *I. spiculatus* uses lotic systems during the dry season to reproduce, a consistent pattern among the forest toad group (Mendelson et al. 1999). With this new data, the information on reproduction sites for the ten species of this group is now complete (Mendelson 2011). Reproduction in lotic environments during the dry season may be related to the flow conditions of the streams, since this is the time when these systems have slower currents and are shallower. Thus, the eggs are not washed away and the drying of water bodies does not represent a risk for the larvae (Kam et al. 1998; Wells 2007).

Based on these field observations, *I. spiculatus* is opportunistic and largely insectivorous in its feeding habits. The feeding strategy of this species is that of a sit-and-wait predator. Even though the sample size for the adult diet analysis was small, it represents the first data on the diet of this species, which is composed of arthropods, including ants, beetles, crickets, and scorpions. Probably the most noteworthy observation is the evidence of scorpion consumption, which is poorly documented in amphibians. While basically restricted to the Neotropics, all reports of scorpions in anuran diets are limited to eight species within three families (Bufonidae, Hylidae, and Leptodactylidae): *Leptodactylus pentadactylus*, *Leptodactylus bolivianus*, *Leptodactylus fuscus*, *Osteopilus septentrionalis*, *Boana pugnax*, *Peltophryne peltoccephalus*, *Rhinella marina* (Botero-Trujillo 2006; Flórez and Banco-Torres 2010), and *Rhinella icterica* (Jared et al. 2020). The recent report of *R. icterica* as a natural predator of the Yellow Scorpion (*Tityus serrulatus*) is interesting, as this scorpion is known for a significant number of poisoning

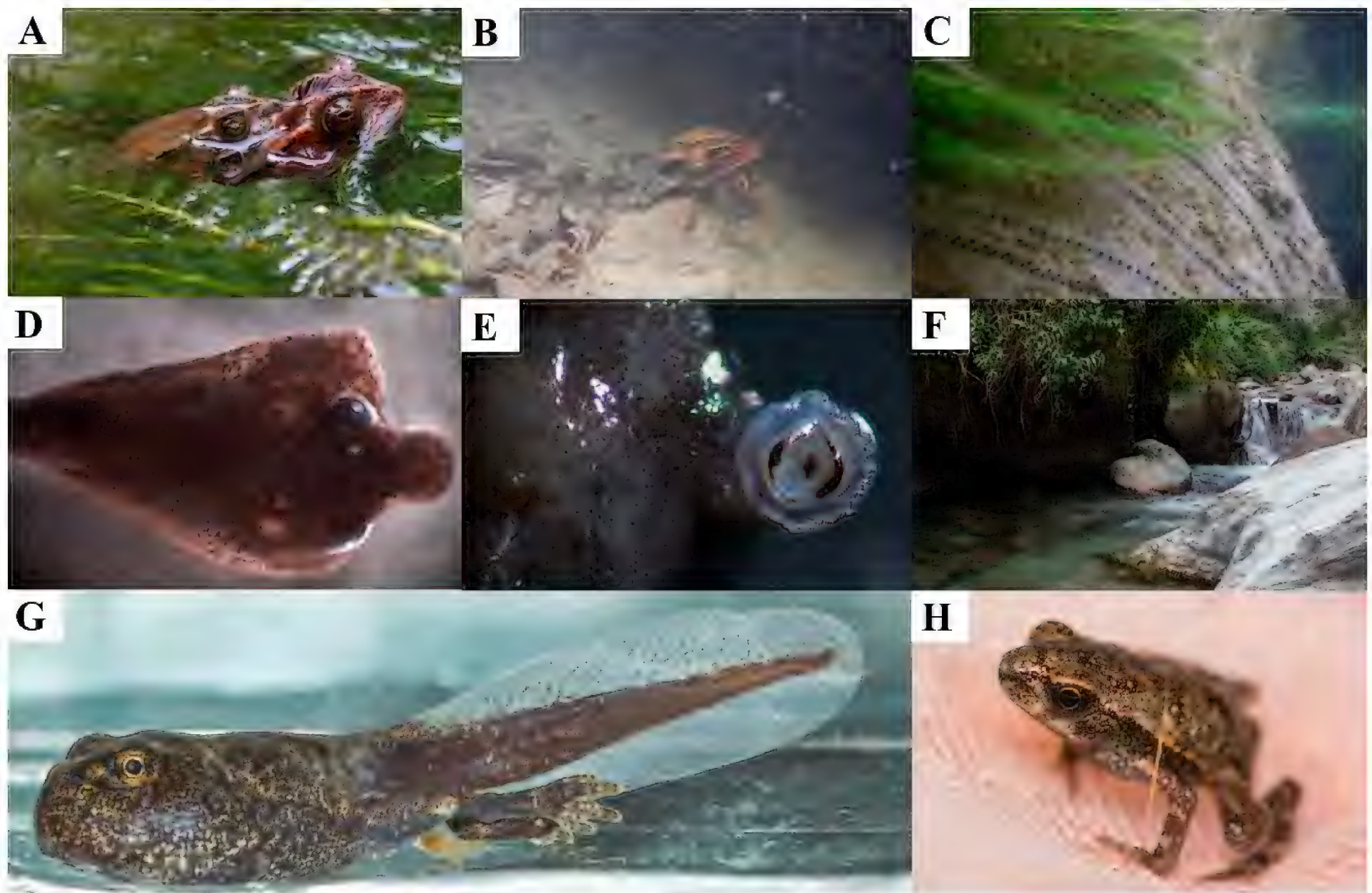


Fig. 2. Breeding behavior of *Incilius spiculatus*. (A–B) Amplexus (axillary type) and oviposition, (C) egg string staggered in unilayered tube, (D–E) dorsal and ventral views of the tadpole head at Gosner stage 24 showing the “elongated mouth,” (F) Rio Coyul, San Pedro Yolo; (G) lateral view of tadpole at Gosner stage 39, and (H) lateral view of metamorphic individual.

incidents involving humans in Brazil (Jared et al. 2020). A broader dietary analysis of *I. spiculatus* should be undertaken in order to compare possible differences between ages and sexes, with the aim of determining different roles in the ecosystem as proposed for other toad species. For example, preferred prey size is related to body size and age in *I. cristatus*: juveniles consume smaller prey than adults, while females, being larger in size, consume larger prey items, suggesting different predator-prey interactions according to age (Gelover et al. 2001; Oropeza-Sánchez et al. 2018).

Despite the fact that the elevational distribution of this species is now extended from 642–1,758 m asl, potential reproductive streams are located at lower elevations. As lowlands present warmer climates, fertile soil, and less slope, they are usually attractive for agriculture and human settlements (Price and Butt 2000; Slik 2005), which results in clearing and deterioration of vegetation cover, especially in riparian areas. These activities contribute to connectivity loss between forest habitat and breeding sites, creating a habitat split that is particularly problematic and risky for amphibians with aquatic larvae (Becker et al. 2007; Price and Butt 2000; Velasco-Murguía et al. 2014). As in other species, this has several implications for the conservation of *I. spiculatus*. First, reproductive adults and metamorphic individuals emerging from the river are forced to migrate between forested areas and lotic environments via disturbed sites, increasing the risks of desiccation

and predation (Oropeza-Sánchez et al. 2018; Todd et al. 2009; Walston and Mullin 2008). Additionally, tadpoles developing in the river have higher exposure to the chemicals used in agriculture and other human activities, such as the extraction of sand and water (Adlassnig et al. 2013; Sparling et al. 2001). These aspects provide additional stress factors to the already vulnerable situation of amphibians, such as *I. spiculatus*, which have small distribution areas, aquatic reproduction, and larval development in lotic systems, making them more susceptible to habitat transformation and increasing their risks of population decline or extinction (Becker et al. 2007; Nowakowski 2017).

Increased efforts to describe anuran larval stages are needed in order to complete our knowledge of the biology of threatened amphibian species. Firstly, this is necessary to understand the habitat requirements of a species, and possibly differential stressor factors throughout the life cycle. Secondly, because tadpoles remain in water bodies for long time periods, they are often the only evidence for the presence of amphibians at many sites, making this life-history stage highly relevant to rapid inventories that aim to accurately assess the geographic distribution of this group. For this reason, the morphological description of tadpoles allows detection of species that are more difficult to detect in their adult phase, while reducing the costs and technical complications of inventories that involve molecular techniques such as bar coding (Grosjean et al.

Key to the tadpoles of the forest toads of Mexico and Central America

1a. A2 Gap present.....4
 1b. A2 Gap absent.....2
 2a. Tail fin coloration variable; known from Mexico.....3
 2b. Tail fins light brown with widely dispersed dark brown dots; known from western Costa Rica and Panama.....
*I. aucoinae*
 3a. Known from Sierra Madre Oriental of Veracruz and Puebla, Mexico.....*I. cristatus*
 3b. Known only from Sierra de los Tuxtlas Veracruz, Mexico.....*I. cavifrons*
 4a. Tail fins transparent.....5
 4b. Tail fins uniformly dark brown.....*I. tutelarius*
 5a. Tail musculature black.....6
 5b. Tail musculature brown.....7
 6a. Tail musculature partially black with scattered pale areas. Known from southern Mexico to western Guatemala.....*I. macrocristatus*; *I. aurarius*
 6b. Tail musculature black. Tail fins reticulated and flecked with black. A-2 gap width about 2 labial teeth. Known only from north-eastern Honduras.....*I. leucomyos*
 7a. Dorsal fin has large Cinnamon Brown (43) granules forming a reticulation. A-2 gap width equal to 3–8 labial teeth. Known only from Sierra Madre de Oaxaca.....*I. spiculatus*
 7b. Dorsal and ventral fins with yellow reticulation. A-2 gap wide, width equal to 10–15 labial teeth. Widely distributed across southern Mexico and Central America.....*I. valliceps*

2015). In addition, morphological data will be useful and complementary to new and powerful techniques, such as environmental DNA, for biodiversity inventories in the coming decades (Beng and Corlett 2020).

A report by the IUCN (IUCN SSC 2020) states that *I. spiculatus* is not distributed within protected areas. However, in our study area, the local community conserves large areas of the montane cloud forest that is habitat for *I. spiculatus* (9,570 ha in the case of the municipality of San Felipe Usila) under the Indigenous and Community Conserved Areas (ICCAs) mechanism. ICCAs are areas governed by indigenous or local communities where collective action focuses on the governance of common resources at multiple scales (Bray et al. 2012; Pazos-Almada and Bray 2018). These social actions contribute to the maintenance of optimal habitat where the species can still survive. Communication with the local community to share findings on reproductive behavior should increase the chances of improving habitat quality at lower elevation sites, promote connectivity between aquatic and terrestrial habitats, and ensure the survival of this species.

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